Nuclease

Week 11

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Inoculation of remaining BL21(DE3) liquid culture containing pSB1C3-T7-Nuc plasmid for IPTG induction with protein extraction and SDS-PAGE analysis

Responsible

Oscar He, Ellinor Lindholm, Oskar hman, and Oscar Frisell

Protocols used

Protein expression
Sonication
SDS-PAGE

Modifications and comments to protocols

	Nuc 2.1.2 GS BL21(DE3) 3	no IPTG
Protein expression (samples):	Nuc 2.1.2 GS BL21(DE3) 3	0.5 mM IPTG
	Nuc 2.1.2 GS BL21(DE3) 3	1.0 mM IPTG

SDS-PAGE gel: Mini-PROTEAN®TGX™ precast gel

Experimental Setup

Table 1: Volumes of each liquid culture. From some, 1 ml was taken for optical density measurement at 660 nm.

Sample	Volume [ml]
Nuc 2.1.2 GS BL21(DE3) 3 - no IPTG	20
Nuc 2.1.2 GS BL21(DE3) 3 - 0.5 mM IPTG	20
Nuc 2.1.2 GS BL21(DE3) 3 - 1.0 mM IPTG	20

When extracting the protein, both the cell debris pellet (resuspended) and the supernatant after sonication and centrifugation is kept and analysed. By investigating the presence of nuclease in both pellet and supernatant, it can be determined whether the protein is soluble (present in the supernatant) or insoluble (present in the pellet).

Results and Conclusions

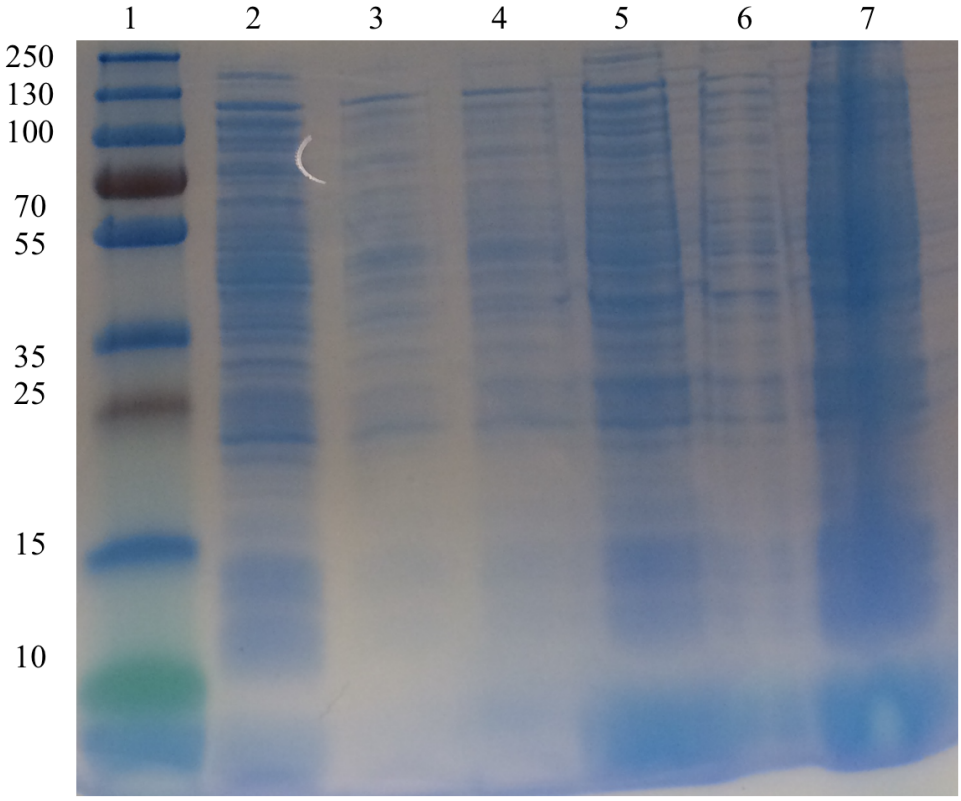

Figure 1: Result of the SDS-PAGE run at 200 V, 3.00 A. (1) Protein ladder (2) Nuc 1.0 mM supernatant (3) Nuc 0.5 mM supernatant (4) Nuc 0.5 mM supernatant (5) Nuc 1.0 mM pellet (6) Nuc 0.5 mM pellet (7) Nuc 0 mM pellet.

A band approximately at the expected size (20.7 kDa) can be seen in well 2 and slightly more faint in well 5.

Discussion and Troubleshooting

The visible band at the expected size, corresponding to nuclease, in well 2 is not present in the wells (3 and 4) with the samples with lower IPTG induction concentration. This indicates that the plasmid of interest is functional and that nuclease is expressed when inducing with IPTG. Since the same band is also visible in well 5 (pellet resuspension sample), and increasingly faint in well 6 and 7 as the IPTG concentration decreases, it is difficult to draw any conclusions whether nuclease is soluble or not. The solubility and the nuclease activity on biofilms will be further tested in a Kirby-Bauer test and a Biofilm assay.

2 Kirby-Bauer test of expressed Nuclease

Responsible

Oscar He, Ellinor Lindholm and Oskar Ohman

Protocols used

Kirby-Bauer test

Modifications and comments to protocols

Protein/antibiotic samples:	Chloramphenicol	20 mg/ml (positive control)
	Nuc 2.1.2 GS BL21(DE3) 3	no IPTG (negative control)
	Nuc 2.1.2 GS BL21(DE3) 3	0.5 mM IPTG
	Nuc 2.1.2 GS BL21(DE3) 3	1.0 mM IPTG
Incubation time	16 h	
Volume of TOB1 cells on McConkey plates	100 μl	

Experimental Setup

The samples were applied in duplicates on two different plates.

Results and Conclusions

The growth of the TOB1 cells were uneven and difficult to interpret.

Discussion and Troubleshooting

A new Kirby-Bauer test will be performed since the results from this one were not reliable.